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Characterization of Passionfruit Crinkle Potyvirus- a Newly Found Virus Infecting Passionfruit

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ABSTRACT

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A virus, designated as passionfruit crinkle potyvirus (PCV), was isolated from passionfruit cultivar Tainung No. 1 causing crinkle symptom on the foliage, different from those induced by the passionfruit mottle (PaMV) and passionfruit woodiness potyviruses (PWV). Unlike PaMV and PWV, PCV did not produce any visible symptom on the fruits. It was identified as a potyvirus on the basis of particle morphology, aphid transmissibility, and the ability to induce cytoplasmic cylindrical inclusions (CI) in infected cells. Of thirty six species in six plant families inoculated, PCV infected 15 species of Passifloraceae, Chenopodiaceae, Leguminosae, Solanaceae, and Amaranthaceae, but all the species tested in Cucurbitaceae were not infected by PCV, indicating that it had wider host range than PaMV and PWV. In light microscopy, large aggregates of bundle-shaped cylindrical inclusions, similar to those induced by PaMV were found accumulated in the cytoplasm of PCV-infected cells. Purified virions and the cylindrical inclusion protein (CIP) were obtained by equilibrium centrifugation in Cs_2SO_4 and preparative electrophoresis, respectively. As analysed in sodium dodecyl sulfate (SDS)

electrophoresis, the relative molecular mass for the capsid and CIP monomer of PCV were estimated to be 35 and 79kDa, respectively. In reciprocal SDS-immunodiffusion and ELISA tests, PCV was found serologically indistinguishable to a potyvirus isolate originating from passionfruit in Puerto Rico, related to, but distinct from, PWV and peanut stripe potyvirus, while it was unrelated to PaMV and 11 other potyviruses.

(Key words: passionfruit crinkle potyvirus, passionfruit, virus, virus identification, potyvirus)

INTRODUCTION

Passionfruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) is a fruit crop widely grown in subtropical regions. In Taiwan, passionfruit is mainly used for juice processing but parts of the production are consumed as fresh fruit. The most widely grown cultivar on the island is 'Tainung No.1' (TN1) which is a F1 hybrid of purple passionfruit (*P. edulis* Sim.) and golden passionfruit (*P. edulis* Sims. f. *flavicarpa* Deg.) bred and officially released by Taiwan Agricultural Research Institute in 1980. The most favorable characteristic for TN1 passionfruit is its self-compatibility between pollen and ovule of the same flower. Therefore, artificial pollenation required for growing golden passionfruit is no longer necessary when TN1 is grown. However, unlike golden passionfruit, TN1 can only be propagated by vegetative means such as grafting but not by seeding. This property led to a heavy outbreak of virus diseases in 1983 due to virus infection on the TN1 mother stock[4,8]. Passionfruit woodinesss virus (PWV) was the first virus detected in passionfruit in Taiwan[12]. Besides PWV, cucumber mosaic virus (CMV) was also found sporadic in Taiwan but caused no evident damage[4,8]. Another potyvirus, namely passionfruit mottle virus (PaMV) was detected in 1987 and found to be as widely spread as PWV on the island [4,6]. PWV induces systemic mosaic and rugose foliar symptoms and produces severely malformed and woody fruits on TN1 but PaMV developed only mild mottling leaf symptoms but no woody and misshaped fruits[4]. In 1989, we isolated from a private passionfruit nursery in central Taiwan another potyvirus designated herein as passionfruit crinkle virus (PCV) on the basis of the unique crinkle foliar symptoms it induced on TN1 passionfruit. Besides biological properties, there were evident antigenic distinctions of PCV from PWV and PaMV[10]. We also found that a virus isolate collected from passionfruit in Puerto Rico (PRPV)[1] had indistinguishable serological properties with PCV. This paper presents the results of characterization on the properties of PCV and compared them with those of the previously described passionfruit potyviruses.

MATERIALS AND METHODS

Viruses

PCV isolate was collected from a TN1

mother stock plant showing crinkle foliar symptom in a private passionfruit seedling nursery in Yuan-Lin[10]. Pure virus culture of PCV was obtained by three successive single lesion transfers through *C. amaranticolor* Coste and Reyn and back inoculated to golden passionfruit seedlings. Infected plants were maintained in separate insect proof cages in greenhouse. PWV and PaMV isolates used for comparison were maintained in the screenhouse as reported previously[4]. PRPV culture[1] was given by F. W. Zettler as dried leaf tissue. It was then propagated on golden passionfruit and maintained similarly as PCV.

Host-range tests

Host-range trials were conducted by mechanical inoculation. At least four plants of each species or cultivar were tested. Inoculum was prepared by grinding leaf tissues with potassium phosphate buffer (100 mM, pH 7.0) in a mortar. Inoculated plants were kept in a screenhouse for at least one month for observation. Virus infection was determined by symptom expression, back inoculation of test plants to golden passionfruit seedlings and by serological tests.

Aphid transmission tests

Aphid transmission trials were conducted with green peach aphids (*Myzus persicae* Sulzer) as previously described[4]. The aphids were reared on tobacco (*Nicotiana tabacum* L. cv. Van-Hicks) and fasted for a 2 hr period before use in transmission tests. A total of twenty golden passionfruit seedlings were used in the transmission test.

Electron and light microscopy

Virus particles of PCV in crude sap of golden passionfruit were negatively stained in 2% potassium phosphotungstate (PTA), pH 6.5, containing 0.1% (W/V) bovine serum albumin[14] and examined with a Hitachi 7000 electron microscope. Epidermal strips of healthy or PCV-infected golden passionfruit leaves were stained either with calcomine orange and "Luxol" brilliant green (O-G) or with Azure A as described by Christie and Edwardson[13] and examined by light microscopy for virus-induced inclusions.

Purification of virus and cylindrical inclusion protein

PCV was propagated in golden passionfruit and maintained in a constant 28 C growth chamber. Leaf tissue collected 14 days post inoculation was used for purifying PCV virions and cylindrical inclusions. The procedures described previously for purification of PWV and PaMV virions and cylindrical inclusion protein (CIP) were followed[4].

Antiserum preparation

Immunization scheme for preparation of antisera against intact virions or virus-induced CIP of PCV was carried out as previously described[4]. Antisera collected 1-3 months after the last injection were used for serological comparisons.

Serological tests

Serological relationships of PCV with other potyviruses were studied by direct ELISA[15] and SDS-immunodiffusion tests [19]. Antigens in infected plant extracts and antisera against the following viruses were used in ELISA tests: bean common mosaic (BCMV)[11], bean yellow mosaic (BYMV)

(9), blackeye cowpea mosaic (BlCMV)(2), cucumber mosaic (CMV)(2), maize dwarf mosaic (MDMV)(16), papaya ringspot type W (PRV-W)(20), PaMV(4), peanut stripe (PStV)(11), potato virus Y (PVY)(19), PRPV (1), PWV(4), soybean mosaic (SMV)(3), watermelon mosaic 2 (WMV-2)(20), and zucchini yellow mosaic (ZYMV)(18). To study further the serological relationships among the potyviruses originated from passionfruit, indirect ELISA as previously described(9) was also performed and the results were compared with those obtained from direct ELISA test.

In SDS-immunodiffusion test, the reactants were arranged so that homologous antigens were placed in wells adjacent to the heterologous ones for comparison. Antigens and antisera of the following potyviruses, not tested in ELISA, were included: clover yellow vein (CYVV)(9), papaya ringspot virus type P (PRV-P)(20), pea seed-borne mosaic (PSBMV)(5), peanut mottle (PMoV) (7), and tobacco etch (TEV)(19).

Electrophoretic analyses of viral proteins

Purified viral proteins were analysed by discontinuous SDS-polyacrylamide gel electrophoresis as described previously(4).

RESULTS

Host range and symptomatology

Of thirty six species in six plant families inoculated with PCV, 15 species in Passifloraceae, Chenopodiaceae, Leguminosae, Solanaceae, and Amaranthaceae were infected. The virus was not able to infect all of the five species tested in Cucurbitaceae (Table 1). Five out of six species of Passifloraceae were infected by PCV and developed systemic foliar mottling symptoms except TN1 which produced only crinkle symptom on the leaves (Fig.1). No mosaic or other evident foliar symptoms as reported for other potyviruses infecting passionfruit was observed throughout the 1 year observation period. Growth vigor, fruit size and shape of PCV-infected TN1 passionfruit was also found indistinguishable from those of healthy plants. Besides Passifloraceae, PCV infected many species of Leguminosae and produced pronounced systemic mottle or mosaic symptoms. In Solanaceae, *Nicotiana benthamiana* Domin. was the only species infected by the virus.

Electron and light microscopy

Flexuous rod-shaped particles were consistently observed in negatively stained PCV-infected plant tissue extracts by electron microscopy (Fig. 2a). Particles similar in shape and size were also observed in samples prepared after virion purification. The mean length of PCV particles was estimated to be 724 nm by measuring 96 virus particles from TN1 leaf dips.

Under light microscopy, cytoplasmic cylindrical inclusions (CI) were readily seen as large witches'-broom liked structures in PCV-infected passionfruit epidermal cells subjected to O-G staining (Fig. 2b). Usually, larger or more CI aggregates could be observed in tissues with a longer post-infection period than in newly infected ones. Similar type of CIs were also seen in epidermal tissues of PCV-infected *N. benthamiana*. No nuclear or amorphous inclusions were observed in PCV-infected tissues stained either with O-G or with Azure A.

Table 1. Host range and reactions of passionfruit crinkle virus in Taiwan

Test Plant	Reactions[1)]	Recovery[2)]
Passifloraceae		
Passiflora edulis	m	+
P. edulis f. sp. *flavicarpa*	M	+
P. edulis × *P. edulis* f. sp. *flavicarpa*	m, C	+
P. suberosa	m	+
P. incarnata	-	-
P. wormingii	m	+
Chenopodiaceae		
Chenopodium quinoa	SLI	+
C. amaranticolor	CL	+
Leguminosae		
Pisum sativum cv. 'Alaska'	-	-
P. sativum cv. 'Taichung # 11'	-	-
Phaseolus vulgaris cv. 'Top Crop'	-	-
P. vulgaris cv. 'Sutter Pink'	M	+
P. vulgaris cv. 'Dubbele Witte'	M	+
P. vulgaris cv. 'Black turtle'	-	-
P. vulgaris cv. 'Black turtle soup'	-	-
P. vulgaris cv. 'Great northern UI 31'	-	-
P. vulgaris cv. 'Stringless green refugee'	-	-
Vigna radiata	-	-
V. mungo	-	-
V. unguiculata ssp. *unguiculata*	SLI	+
V. unguiculata ssp. *sesquipedalis*	-	-
V. angularis	m	+
Glycine max	m	+
Arachis hypogaea cv. 'Tainung No. 4'	-	-
Cassia occidentalis	m	+
Solanaceae		
Nicotiana benthamiana	m	+
N. tabacum cv. 'Havana'	-	-
N. debnyii	-	-
Lycopersicum esculentum	-	-
Datura stramonium	-	-
Amaranthaceae		
Gomphrena globosa	SLI	+
Cucurtibaceae		
Cucumis sativus	-	-
C. melo	-	-
Citrullus vulgaris	-	-
Momordica charantia	-	-
Luffa cylindrica	-	-

1) M: Mosaic; m: mottle; SLI: symptomless infection; C: crinkle; -: no infection.

2) Recovery tests were done one month after inoculation by back-inoculation on *C. amaranticolor*.

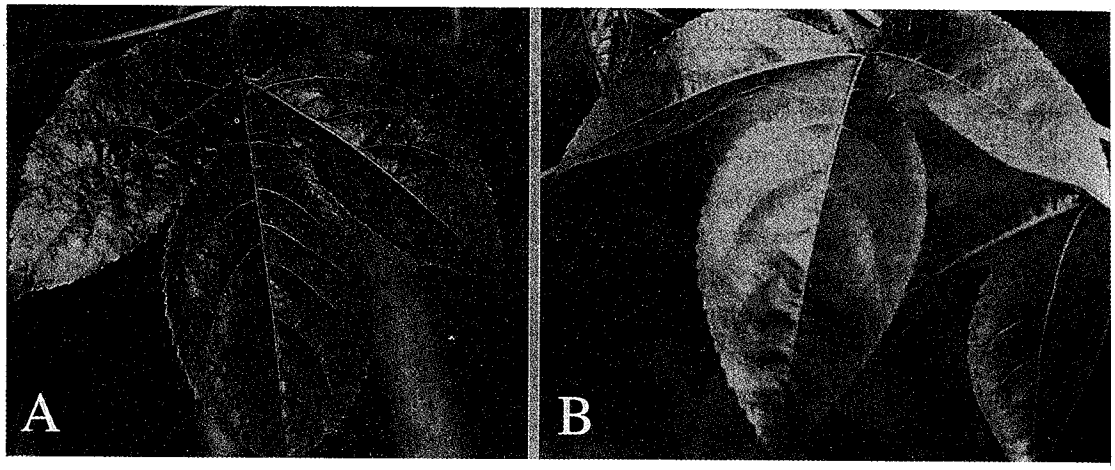

Fig. 1. Foliar crinkle symptom (A) on passionfruit cultivar Tainung No. 1 (TN1) induced by passionfruit crinkle virus as compared to the healthy shining appearance of uninoculated TN1 leaf (B).

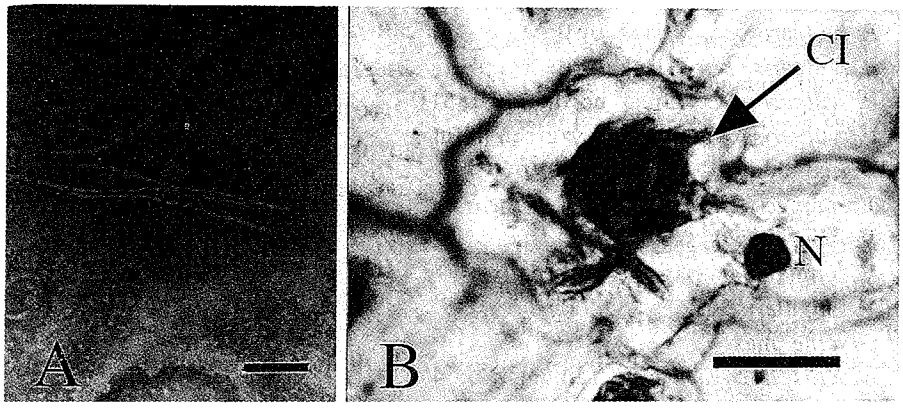

Fig. 2. (A) Electron micrograph of negatively stained virus particles of passionfruit crinkle virus (PCV). Bar represents 200 nm in length. (B) Light micrograph of PCV-infected epidermal tissue of golden passionfruit stained with Luxol brilliant green and calcomine orange showing bundle-shaped cylindrical inclusions (CI) accumulated beside the nucleus (N). Bar represents 10 um in length.

Aphid transmission trial

A total of 20 golden passionfruit seedling tested, 18 became infected.

Purification of virus and viral proteins

Purified samples of PCV had ultraviolet absorption spectrum typical of potyviruses with maximum and minimum absorption at 260 and 247 nm, respectively. Without light scattering correction, the optical density ratio (260/280) was 1.23. The estimated yield of purified PCV was about 1.5 mg per 100 g of golden passionfruit leaves, using an extinction coefficient of 2.4 for potyviruses. Purified virions and

CIP were analysed in 12% polyacrylamide gels to determine their purities and protein species. Purified samples of PWV and PaMV were included for direct comparisons (Fig. 3). All three viruses had a single species of protein monomer for CP and CIP. No contaminant proteins were detected in the purified samples. The relative molecular mass (Mr.) for CPs of PaMV, PWV and PCV were estimated as 37, 36 and 35 kDa, and for CIPs as 79, 76 and 79 kDa, respectively.

Serology

Antisera against intact virions and electrophoresis-purified CIP of PCV were prepared. Serological relationships between PCV and other potyviruses were studied by SDS-immunodiffusion and ELISA tests. Of 15 different potyvirus antigens that were tested in SDS-immunodiffusion, only PRPV, a potyvirus isolated from passionfruit in Puerto Rico reacted with antiserum against PCV virion (Fig. 4). When antiserum against PCV CIP was used, antigens

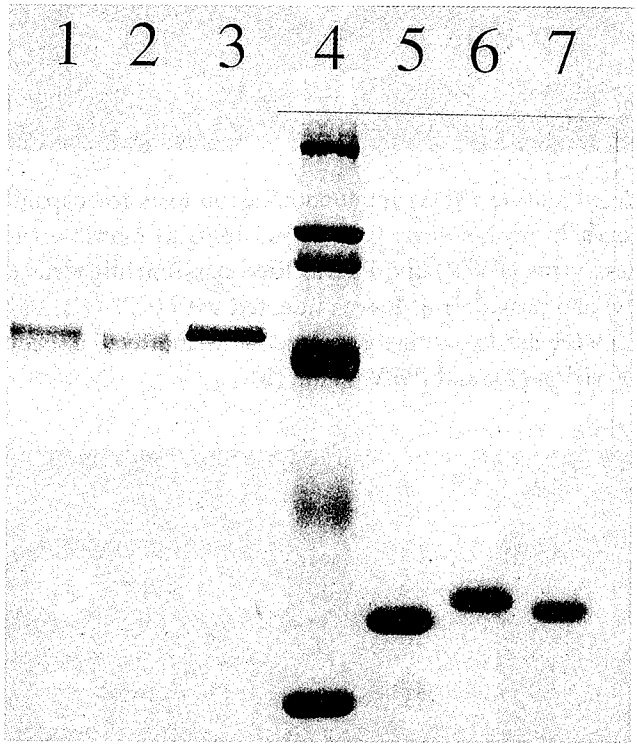

Fig. 3. Analysis of purified capsid (CP) and cylindrical inclusion proteins (CIP) induced by passionfruit crinkle virus (PCV), passionfruit woodiness virus (PWV) and passionfruit mottle virus (PaMV) by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Lane 1, purified PaMV CIP with estimated relative molecular mass (Mr) of 79 kDa; lane 2, purified PWV CIP of 76 kDa; lane 3, purified PCV CIP of 79 kDa; lane 4, six marker proteins of (from bottom to top) carbonic anhydrase (29 kDa), ovalbumin (45 kDa), bovine serum albumin (66 kDa), phosphorylase B (98 kDa), galactosidase (116 kDa), and myosin (200 kDa); lane 5, purified PCV CP of 35 kDa; lane 6, purified PaMV CP of 37 kDa; lane 7, purified PWV CP of 36 kDa. .

of PRPV and PWV, but not the rest 13 different viruses, were reacted positively (Fig. 5). The two antisera were not able to detect any serological difference between PCV and PRPV, however, they readily distinguished PCV from PWV (Fig. 4, 5). The following potyvirus antigens did not react with PCV virion antiserum nor did with the PCV CIP antiserum: BCMV, BlCMV, BYMV, CYVV, PaMV, PSbMV, PStV, PMoV, PWV, TEV, ZYMV, PRV-W, PRV-P, and PVY. In reciprocal tests, among 14 different antisera used, only those against PWV and PStV virion reacted against PCV antigen with spur formation (Fig. 4, Table 2). To discern further the

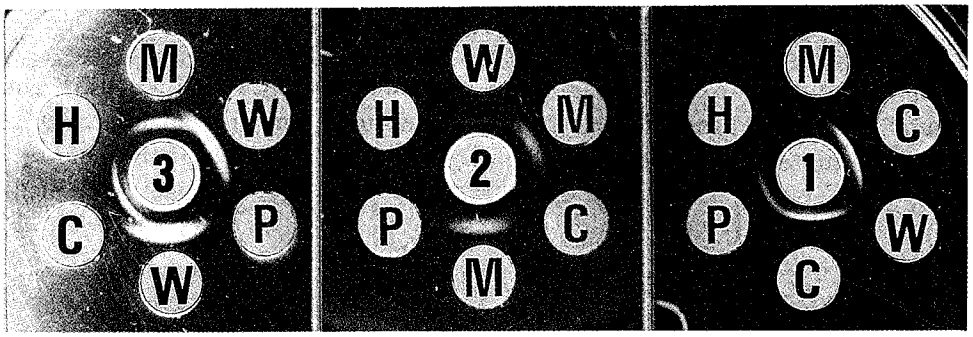

Fig. 4. Sodium dodecyl sulfate (SDS) immunodiffusion tests for capsid protein antigenicities of passionfruit crinkle virus (PCV), passionfruit mottle virus (PaMV), passionfruit woodiness virus (PWV) and Puerto Rico passionfruit virus (PRPV). SDS-treated healthy (H) and passionfruit leaves infected with PCV (C), PaMV (M), PRPV (P), or PWV (W) were used as antigens to react with undiluted antisera against PCV virion (1), PaMV virion (2), and PWV virion (3).

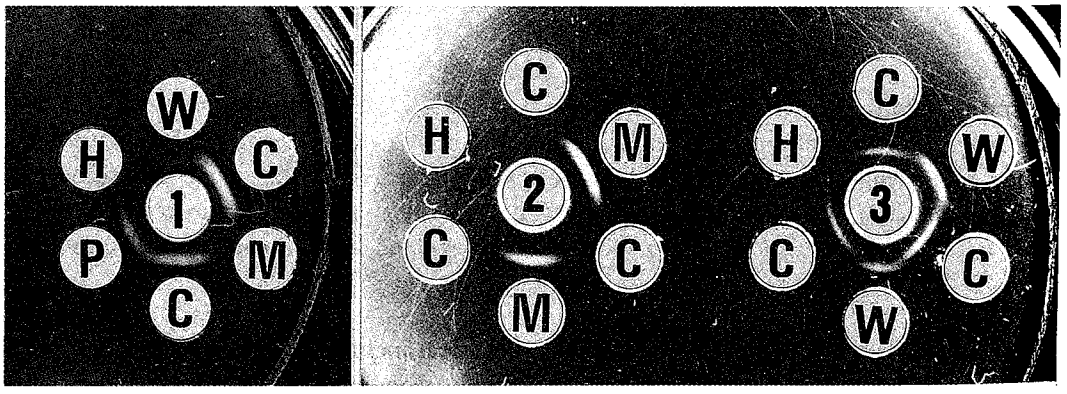

Fig. 5. Sodium dodecyl sulfate (SDS) immunodiffusion tests for cylindrical inclusion protein (CIP) antigenicities of passionfruit crinkle virus (PCV), passionfruit mottle virus (PaMV), passionfruit woodiness virus (PWV) and Puerto Rico passionfruit virus (PRPV). SDS-treated healthy (H) and passionfruit leaves infected with PCV(C), PaMV (M), PRPV (P), and PWV (W) were used as antigens to react with undiluted antisera against PCV CIP (1), PaMV CIP (2), and PWV CIP (3).

serological relationship between PCV, PWV, PaMV, PRPV and PStV, antisera against CIPs of PaMV, PWV and PStV were included in the test and the results are summarized in Table 2. Only the antiserum against PWV-induced CIP reacted with PCV antigen, but the homologous precipitation lines clearly spurred over the heterologous ones (Fig. 5), confirming that although PWV was antigenically related to, but still distinguishable from, PCV. PStV, however, was only distantly related to PCV by sharing some common antigenicities in CP but not in CIP. PaMV, on the other hand, was apparently different from PCV in both the antigenicities of CP and CIP.

Table 2. Serological relationships of PCV with two known passionfruit viruses found in Taiwan and with some other potyviruses as determined by SDS-immunodiffusion test [1)]

Antiserum [4)]	SDS-treated antigens [2,3)]						
	PCV	PRPV	PaMV	PWV	PStV	BlCMV	BCMV
PCV	I	I	-	-	-	-	-
PCV-CI	I	I	-	S	-	-	-
PaMV	-	-	I	S	-	-	-
PaMV-CI	-	-	I	S	-	-	-
PWV	S	S	S	I	-	-	-
PWV-CI	S	S	S	I	-	-	-
PStV-Ts	S	S	S	S	I	*[5)]	*
PStV-Ts-CI	-	-	-	-	I	*	*
BlCMV	-	-	S	S	*	I	*
BCMV	-	-	S	S	*	*	I

1) SDS-immunodiffusion tests were done as described by Purcifull and Batchelor (1977).

2) BCMV = bean common mosaic virus isolate US1 from USA; BlCMV = blackeye cowpea mosaic virus isolate T from Taiwan; PaMV = passionfruit mottle virus from Taiwan; PCV = passionfruit crinkle virus from Taiwan; PRPV = Puerto Rico passionfruit virus from Puerto Rico; PStV = peanut stripe virus isolate Ts from Taiwan; PWV = passionfruit woodiness virus from Taiwan.

3) Results of cross-reactivities are the reactions between homologous and heterologous antigens: I = precipitin lines of homologous and heterologous reactions fused, without spur formation; S = homologous reactions spurred over heterologous reactions; - = no reaction.

4) Antiserum indicated with virus names followed by -CIP are those against cylindrical inclusion protein of the viruses. Antisera against WMV-2 and ZYMV were kindly provided by D. E. Purcifull. All other antisera were produced in our laboratory.

5) Not tested in this study.

Further evidence showing antigenic distinction between PWV and PCV was obtained when antisera against BCMV and BlCMV were found to react with PWV, but not with PCV (Table 2).

In direct (double antibody sandwitched) ELISA tests, none of the 11 antisera against BCMV, BlCMV, BYMV, MDMV, PaMV, PStV, PWV, PVY, SMV, ZYMV, and WMV2 reacted with PCV antigen (Table 3) . To further study PCV's serological relationship with other potyviruses especially those originated from passionfruit, we conducted both direct and indirect (antigen coated type) ELISA using antiserum against PCV virion and the results are shown in Fig. 6. Both tests indicated that PCV and PRPV were serologically indistinguishable. Similar to the SDS-immunodiffusion test results, PWV was found serologically related to, but distinct from, PCV, while PaMV and PCV were evidently serologically unrelated.

Cross protection test

To reveal the biological relationships among the potyviruses isolated from passionfruit in Taiwan, we performed a cross protection test by immunizing TN1 passionfruit in advance with PCV and challenging afterward with PWV and PaMV. Cross protection effect was recognized by the mild crinkle symptom maintained on the test plants, while the failure of cross protection would be the appearance of PWV and PaMV-specific mosaic and mottle symptoms, respectively. The results in Table 4 indicated that PCV could not cross protect TN1 passionfruit from the infection by PWV and PaMV since the specific severe symptoms appeared consistently about 2 weeks after challenge inoculation either by grafting or by aphid transmission. The uninterrupted multiplication of PWV and PaMV in the PCV-immunized passionfruits was also confirmed by ELISA tests.

DISCUSSION

The newly isolated PCV from Taiwan should be recognized as a potyvirus based on its particle morphology, aphid transmissibility and the ability to induce cytoplasmic CIs as shown in this study. There have been four potyviruses, namely PWV[21], PaMV[4], passionfruit ringspot (PaRV)[17], and PRPV[1] reported to infect passionfruits. Among them, PaRV cultures are no longer available to allow a valid comparison with other recently found potyviruses. However, based on the original report[17], the unique ability of PaRV to incite ringspot symptom on the foliage is apparently different from other reported passionfruit potyviruses including PCV. In addition, PCV could systemically infect many species of Leguminosae including soybean and blackeye cowpea which were shown uninfected with PaRV by De Wijs[17]. Although further studies may be required to conclusively determine the relationships between PaRV and PCV, they are possibly two distinct viruses.

In a previous study, we have extensively compared PWV with PaMV and shown that they are related but distinct potyviruses[4]. In present studies, we demonstrated that PCV had many unique characteristics which clearly differed from those of PWV and PaMV. Most obviously, PCV induced unique crinkling symptoms on the foliage

Table 3. Serological relationships of passionfruit crinkle, passionfruit mottle and passionfruit woodiness viruses to nine other potyviruses as determined by direct ELISA tests [1)]

Antiserum[2)]	Absorbance (A405nm) [3)]									
	Homologous		PCV		PaMV		PWV		Healthy sap	
	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}
PCV	1.10	0.21	-[4)]	-	0.12	0.10	0.19	0.06	0.13	0.03
PaMV	0.95	0.72	0.08	0.10	-	-	0.04	0.03	0.07	0.00
PWV	1.84	1.77	0.10	0.05	0.30	0.12	-	-	0.14	0.02
BlCMV	1.83	1.26	0.08	0.10	0.21	0.20	0.25	0.13	0.08	0.05
PStV-Ts	1.38	1.01	0.08	0.03	0.07	0.04	0.11	0.04	0.09	0.03
SMV	1.92	1.88	0.05	0.04	0.08	0.05	0.15	0.07	0.07	0.05
WMV-2	1.85	0.87	0.08	0.03	0.12	0.11	0.09	0.07	0.12	0.07
ZYMV	1.90	1.59	0.11	0.03	0.06	0.03	0.16	0.02	0.13	0.03
MDMV	1.87	1.85	0.04	0.03	0.03	0.02	0.03	0.02	0.03	0.03
BYMV	1.24	0.83	0.07	0.10	0.09	0.05	0.06	0.04	0.04	0.05
PVY	0.83	0.41	0.03	0.03	0.05	0.00	0.00	0.00	0.09	0.04
BCMV	1.68	1.51	0.05	0.02	0.04	0.02	0.05	0.02	0.08	0.01

[1)] Direct ELISA test was conducted as described in the text. Immunoglobulins (IgG) to each virus were used at a concentration of 1 ug/ml. The alkaline phosphatase conjugated IgGs from each virus antiserum were used at a dilution of 1/1,000. Virus infected tissues were ground in 0.05M phosphate buffer, pH 7.2, at a ratio of 10 ml/g. The sap obtained was taken as a dilution of 10-1.

[2)] BlCMV = blackeye cowpea mosaic, BYMV = bean yellow mosaic, MDMV = maize dwarf mosaic, PaMV = passionfruit mottle, PRV-W = papaya ringspot virus type W, PStV = peanut stripe, PVY = potato virus Y, PWV = passionfruit woodiness, SMV = soybean mosaic, WMV-2 = watermelon mosaic virus 2, ZYMV = zuchinni yellow mosaic. Antisera against PVY, WMV-2, and ZYMV were kindly provided by D. E. Purcifull. All other antisera were produced in our laboratory.

[3)] Absorbance values were an average of three replicate wells.

[4)] Absorbance readings identical to those shown in homologous reactions.

and did not produce any conspicuous symptom, as PWV and PaMV did, on the fruits of TN1 passionfruit. Since TN1 is a F1 hybrid of *P. edulis* and *P. edulis* fsp. *flavi* carpa, *individual seedlings propagated vege* tatively should theoretically share identical genetic background. The ability of PCV to induce unique foliar and fruit symptoms on TN1 suggested that the virus apparently encoded different pathogenicity genes from those of PWV and PaMV. In addition, PCV could systemically infect *Glycine max* (L.)

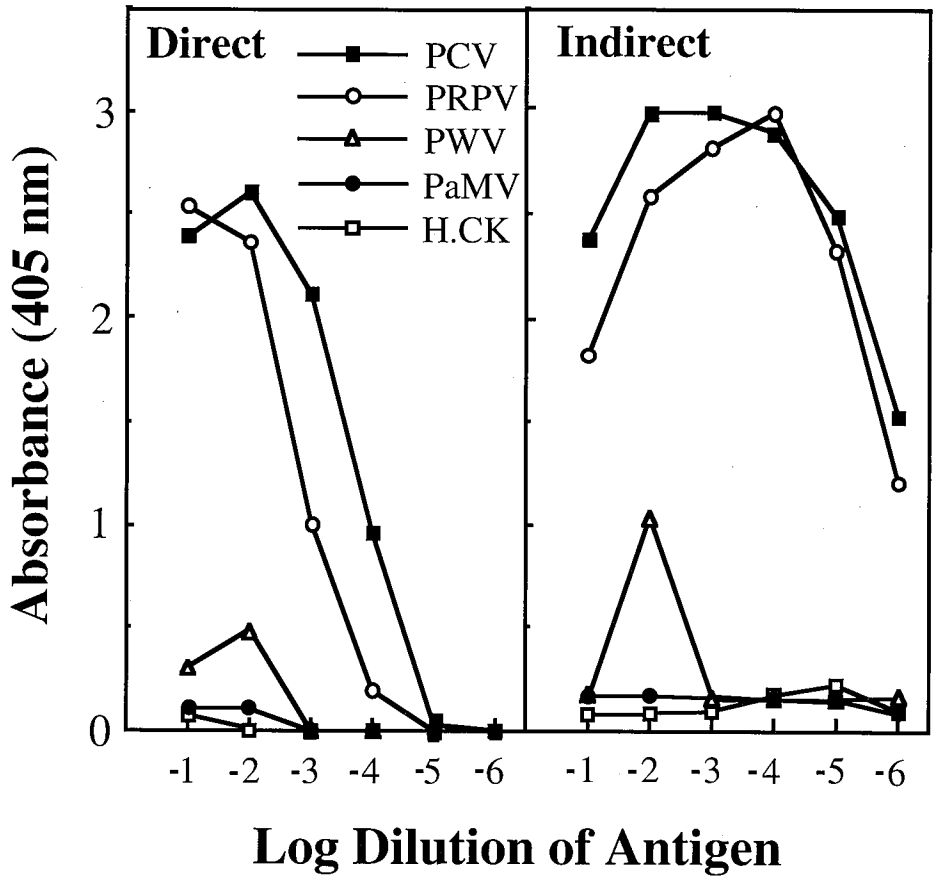

Fig. 6. Serological comparison of passionfruit crinkle virus (PCV), passionfruit mottle virus (PaMV), passionfruit woodiness virus (PWV) and Puerto Rico passionfruit virus (PRPV) by direct and indirect enzyme-linked immunosorbent assay (ELISA). In direct ELISA (left), the procedure described by Clark and Adams (15) was followed and the antigens were diluted with 10 mM potassium phosphate buffer, pH 7.2. In indirect ELISA, antigens were diluted with sodium carbonate buffer, pH 9.0, and subsequently used to coat the plates as previously described (9). Immunoglobulin (IgG) purified from the antiserum against PCV virion collected 2 mo after last immunization was used at a concentration of 1 ug/ml. Goat anti-rabbit IgG alkaline phosphatase (AP) conjugate and AP conjugate of IgG to PCV antiserum used in indirect and direct ELISA, respectively, were both diluted 1/1,000.

Merr. and *Gomphrena globosa* L. which were shown uninfected by PWV and PaMV [4]. On the contrary, our previous study showed that PWV and PaMV could infect *Phaseolus vulgaris* L. cv. 'Black turtle' and 'Black turtle soup'[4] but both were found uninfected by PCV in this study. Furthermore, several serological assays conducted in this study conclusively showed that PCV was unrelated to PaMV, though it was related to, but clearly distinct from, PWV in the antigenicities of CP and CIP. The differ-

ences among these three viruses were also revealed by the disparities of the Mr. of their CP and CIP as determined in SDS-PAGE. Moreover, the failure of PCV to cross protect TN1 passionfruit from the infection by PWV and PaMV provided another strong evidence for PCV's biological uniqueness. On the basis of these results, we believe that PCV should be a distinct potyvirus infecting passionfruit in Taiwan. However, PRPV, a virus isolate from Puerto Rico reported in 1991[1] was interestingly found to be serologically indistinguishable from PCV. Besides serology, PRPV was also found to induce symptoms on TN1 passionfruit similar to those caused by PCV (data not shown). This finding suggests that PRPV and PCV are likely isolates of the same virus. Therefore, we propose that "PCV" should be referred to as the better name for these nynomyms since the naming of PCV[10] predates PRPV[1] and apparently the virus has occurred not only in Puerto Rico.

Since passionfruit is native in the region of southern America, we speculate that PCV may originate from the same area where PRPV was found and be introduced to Taiwan through passionfruit germplasm collection process. Although exchange of germplasm is an inevitable process for crop improvement, without due attention to care on quarantine procedures germplasm exchanges would certainly result in more suffer than benefit for farmers.

Virus diseases of passionfruit in Taiwan have been successfully managed at a level that no serious losses are experienced by growers since the implementation of virus free seedling propagation program in 1988[8]. Occurrence of the newly recognized PCV represents a forthcoming challenge to the passionfruit industry in Taiwan. Fortunately, PCV was only once found from a private passionfruit seedling nursery and has not been found to infect any passionfruit plants in the major production regions since 1989. Although our study did not reveal any detrimental effect of PCV on the growth and production of our current TN1 cultivar, its pathogenic effect on the other cultivars such as golden passionfruit still warrants attention. Besides PaMV and PWV, PCV was further included in 1991 as a target virus in the virus indexing program currently implementing in Taiwan for producing healthy mother stock passionfruit trees. The program has been carrying out for 5 years and proved to be successful in maintaining PCV-free status for the major passionfruit growing regions of Taiwan.

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摘　　要

張清安[1]、陳金枝[1]、鄧汀欽[1]、Zettler, F. W.[2] 1996 百香果漣葉病毒——一種新發現之馬鈴薯Y屬病毒之鑑定 植保會刊 38: 339-354. ([1]台灣省農業試驗所; [2] Dept. of Plant Pathology, Univ. of Florida)

本研究由本省台農一號百香果分離獲得一種與文獻上所記錄之百香果木質化病毒 (passionfruit woodiness potyvirus, PWV) 及百香果斑紋病毒 (passionfruit mottle potyvirus, PaMV) 性質迥異之馬鈴薯Y屬病毒。此病毒株系感染台農一號百香果時僅在葉部產生漣葉 (crinkle) 病徵，對果實之外型與著色並無任何影響。此種病徵與PWV及PaMV所造成者有顯著差異，因此將其定名爲百香果漣葉病毒 (passionfruit crinkle potyvirus) 簡稱PCV。PCV具長絲狀易彎曲型顆粒 (elongated flexuous rods)，可由桃蚜以非永續型方式 (non-persistent manner) 傳播，於感病細胞質中可產生圓柱狀內含體 (cylindrical inclusion)。在所接種之6科36種植物中只有葫蘆科之瓜類植物不能被PCV感染，顯示其寄主範圍較PWV及PaMV寬廣。另外PCV可於大豆 (*Glycine max* (L.) Merr.) 及千日紅 (*Gomphrena globosa* L.) 感染造成系統性病徵之特性也與PWV及PaMV不同。利用交叉保護法亦證實PCV無法保護百香果免於PWV及PaMV之重複感染。以高低速交替離心 (differential centrifugation) 及平衡離心法 (equilibrium centrifugation) 可順利將PCV 病毒顆粒由百香果葉片中純化。純化試料經電泳分析估計其鞘蛋白基本單位 (capsid protein subunit) 之分子量爲35kDa。在純化病毒顆粒之過程中可同時純化出PCV於感病細胞質內所合成之圓柱狀內含體蛋白 (cylindrical inclusion protein, CIP)，其分子量則估計爲79kDa。將純化之病毒顆粒及CIP進行免疫注射於家兔體內已製成專一性抗體。將此等抗體應用於SDS免疫擴散反應 (SDS-immunodiffusion test) 及酵聯抗體法 (ELISA) 測試，發現PCV與PaMV無任何血清類緣關係，而PCV雖與PWV有部份血緣關係但彼此間仍有顯著差異，但PCV 之血清學性質與 Bird *et al.* (1991) 自波多黎各百香果所分離而未充分鑑定之 Puerto Rico passionfruit potyvirus 則完全相同無法區別。另外與其他12種已知之馬鈴薯Y屬病毒比較時，發現PCV只與落花生條斑病毒 (peanut stripe potyvirus, PStV) 有部份血緣關係，而與其他11種病毒無任何血緣關係。由現有證據顯示PCV與過去在百香果上已記錄之數種馬鈴薯Y屬病毒之特性有明顯差異，而與其他已知之病毒亦呈顯著之不同，故應屬一種尚未經充分鑑定之新病毒。

(關鍵詞：百香果漣葉病毒、百香果、病毒、病毒鑑定)